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TWO CASES OF DUODENAL GANGLIOCYTIC PARAGANGLIOMA: IMMUNOCYTOCHEMICAL CHARACTERISTICS

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Abstract Two cases of duodenal gangliocytic paraganglioma were studied by means of immunocytochemical methods using 41 kinds of antibodies. The tumors consisted of three histological types; carcinoid, ganglioneuroma and paraganglioma. Tumors of both cases exhibited immunoreactivity to at least one or as many as three of the following: calcitonin, calcitonin-gene related peptide, endocrine granule constituent, Leu7, neuropeptide Y and basic fibroblast growth factor. In addition, these tumors were also immunopositive for neuron specific enolase, S-100 protein, neurofilament protein, pancreatic polypeptide, chromogranin A, somatostatin, leu-enkephalin, substance P and vasoactive intestinal peptide, as has been described in previous reports. In one case, tumor cells were immunopositive for adrenocorticotropin, bombesin, gastrin releasing peptide, myelin basic protein, neuroendocrine marker and tyrosine hydroxylase. Moreover, paraganglioma cells of tumors showed both argyrophilia and argentaffinity. These results suggest that duodenal gangliocytic paraganglioma may originate from embryonic neuroinsular complex.

Key words: Gangliocytic paraganglioma, immunocytochemistry, neuroendocrine markers, histogenesis

INTRODUCTION

Gangliocytic paraganglioma is an uncommon, largely benign, small intestinal neoplasm that usually occurs in the second portion of the duodenum, rarely in the jejunum^{1,2)} and in the peripancreatic tissue adjacent to large vessels³⁾. This tumor is generally solitary but a case involving multiple tumors has been documented³⁾.

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Immunocytochemical characteristics of 52 cases in the literature²⁻¹²⁾ examined, with a focus on the common and uncommon neuroendocrine features of this tumor. Recently, we had the opportunity to study two cases of this tumor using light microscopy and the streptoavidin-biotin immunostaining technique. On the basis of the results obtained, a possible origin of this tumor is discussed.

CASE REPORT

Case 1: A 48-year-old man was hospitalized for epigastralgia. He had a past history of duodenal ulcer and had undergone gastroduodenectomy 7 years earlier. An X-ray study confirmed the presence of a smooth surfaced mass in the second portion of the duodenum. Endoscopic examination disclosed a tumor mass with an eroded surface of papilla Vateri. Surgical exploration permitted complete excision of the duodenal tumor by combined removal of the duodenum and pancreatic head. The postoperative course was uneventful.

Case 2: A submucosal tumor was found in the second portion of the duodenum of a 51-year-old man by x-ray and endoscopic examination. His chief complaint was epigastric pain. The mass was resected and the diagnosis of the frozen section was gangliocytic paraganglioma. Recovery was uneventful.

MATERIALS AND METHODS

Light Microscopy and Histochemistry

The surgical specimens were fixed in 10% buffered formalin and processed in the usual manner for light microscopic study. Tissue sections were stained with hematoxylin and eosin and were subjected to argyrophilic Grimelius and Fontana-Masson argentaffin analysis.

Immunocytochemistry

Immunocytochemical studies were carried out on the formalin-fixed, paraffin-embedded tissue by the method described by Hsu *et al.*¹³⁾, using a kit of biotin-labelled second antibody and streptoavidin coupled with peroxidase (Nichirei Corp., Tokyo, Japan). The antibodies and abbreviations used are listed in Table I. Anti-bombesin is bombesin specific without any cross reactivity with gastrin-releasing peptide (GRP), nor does anti-GRP antibody react with bombesin¹⁴⁾. The anti-human pancreatic polypeptide (hPP) and porcine PP antibodies used do not recognize neuropeptide Y (NPY) and anti-NPY antibody does not bind to hPP or PPP. This was confirmed by a preabsorption test of the anti-hPP or anti-PPP antibodies with NPY (1 µg/ml, Sigma, St. Louis, MO). Every absorption test revealed positive staining of the tumor cells (data not shown). As the insulin antibody was raised in guinea pig, a guinea pig PAP (peroxidase-antiperoxidase complex) kit (IBL, Gunma, Japan) was used. The peroxidase reaction product was visualized by Graham-

Table 1. List of antibodies used

Category	Antibody to	Source	Working titer
Peptide	Adrenocorticotropin (ACTH)	(Dr. Ito)* ¹	1:200
	Bombesin	(Dr. Yanaihara)* ²	1:1,000
	Calcitonin	(Dr. Yamada)* ¹	1:1,000
	Calcitonin-gene related peptide (CGRP)	(Peninsula)	1:3,000
	Chromogranin A	(Dakopatts)	1:500
	Endocrine granule constituent (EGC)	(MILAB)	1:500
	α -Endorphin	(Dr. Ito)	1:500
	β -Endorphin	(Dr. Ito)	1:500
	leu-Enkephalin	(Dr. Ito)	1:500
	Gastrin	(Dakopatts)	1:5,000
	Glucagon-like immunoreactivity (GLI)	(IBL)	1:500
	Glucagon	(IBL)	1:500
	Gastrin releasing peptide (GRP)	(Dr. Yanaihara)	1:2,000
	Insulin	(Dr. Ito)	1:500
	Neuroendocrine marker	(Lipshaw)	1:1
	Neuropeptide Y (NPY)	(Dr. Ito)	1:500
	Neurotensin	(Dr. Yanaihara)	1:1,000
	Human pancreatic polypeptide (hPP)	(Dr. Ito)	1:200
	Porcine pancreatic polypeptide (PPP)	(Dr. Yanaihara)	1:1,000
	Parathormone	(IBL)	1:500
	Somatostatin	(Dr. Ito)	1:100
	Substance P	(Dr. Yanaihara)	1:1,000
	Vasoactive intestinal polypeptide (VIP)	(Dr. Yanaihara)	1:500
Neuronal marker	Brain natriuretic peptide (BNP)	(IBL)	1:500
	Glial fibrillar acidic protein (GFAP)	(IBL)	1:1,000
	Myelin basic protein (MBP)	(IBL)	1:500
	Neurofilament protein (NFP)	(Boehringer)	
	68 kDa		1:4
	160 kDa		1:4
	200 kDa		1:4
Growth factor	Neuronspecific enolase (NSE)	(IBL)	1:500
	S-100 protein	(IBL)	1:500
	Acidic fibroblast growth factor (a-FGF)	(Dr. Sasaki)* ³	1:500
	Basic fibroblast growth factor (b-FGF)	(Dr. Sasaki)	1:500
Others	Insulin-like growth factor-II (IGF-II)	(Cosmo-Bio)	0.1 μ g/ml
	Carcinoembryonic antigen (CEA)	(IBL)	1:1,000
Others	Epithelial keratin (AE-1, AE-3)	(ICN Immunobiol.)	1:100
	Leu-7	(Becton Dickinson)	0.2 μ g/ml
	Serotonin	(Dr. Yui)* ¹	1:1,000
	Tyrosine hydroxylase	(Dr. Kumanishi)* ¹	1:400

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Karnovsky solution¹⁵⁾ containing 20 mg/dl of 3, 3'-diaminobenzidin tetrahydrochloride (Dojin, Kumamoto, Japan) and nuclei were stained by 1% methyl green. Endogenous peroxidase activity was blocked by 0.3% H₂O₂ in methanol prior to immunostaining and also by 65 mg/dl of sodium azide (Wako Pure Chem. Indust., Osaka, Japan) in Graham-Karnovsky solution.

RESULTS

Light microscopy

Tumors of both patients were well-circumscribed in the duodenal wall, as shown in Fig. 1a, and involved the submucosa, with a focal ulceration in case 1. Three histological patterns were observed in both tumors; paraganglioma, ganglioneur-

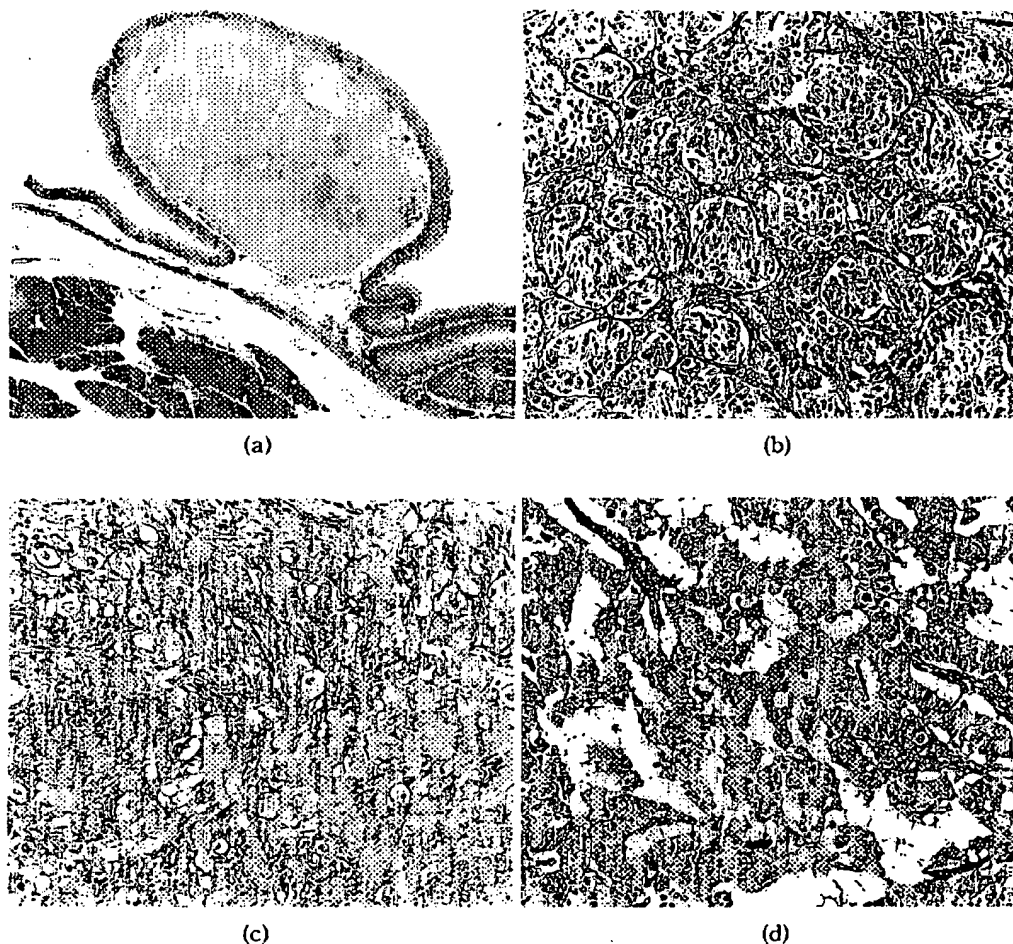


Fig. 1. In case 1, the tumor in the duodenal wall is well-circumscribed and involves the submucosa with focal ulceration of the overlying mucosa (arrowhead, a). Both tumors show three histological patterns: paraganglioma (b), ganglioneuroma (c) and carcinoid (d). a; $\times 5$, b-d; $\times 100$.

oma and carcinoid. In case 1, ganglioneuroma was predominant, whereas in case 2, paraganglioma and ganglioneuroma were equally observed. The carcinoid type was the least represented in both cases. Amyloid, as described in the previous report by Reed *et al.*¹¹⁾, was not present.

The paraganglioma involved the lamina propriae of the duodenal mucosa in both cases and was characterized by a rounded nest of epithelioid cells demarcated

Table 2. Histo- and Immunocyto-chemical Profile of Gangliocytic Paraganglioma

Category	Case		Histological component positively stained
	1	2	
Histochemistry			
Grimelius	+	+	paraganglioma
Masson-Fontana	+	+	paraganglioma
Peptide			
Adrenocorticotropin	—	+	paraganglioma
Bombesin	—	+	paraganglioma
Calcitonin	+	+	carcinoid/ganglioneuroma
Calcitonin gene related peptide	#	#	carcinoid/ganglioneuroma/paraganglioma
Chromogranin A	#	#	carcinoid/ganglioneuroma/paraganglioma
Endocrine granule constituent	#	#	carcinoid/ganglioneuroma/garaganglioma
leu-Enkephalin	#	#	ganglioneuroma/paraganglioma
Gastrin releasing peptide	—	#	carcinoid/paraganglioma
Neuroendocrime marker	—	+	paraganglioma
Neuropeptide Y	#	+	paraganglioma
Pancreatic polypeptide, human	#	+	carcinoid/paraganglioma
Pancreatic polypeptide, porcine	#	+	carcinoid/ganglioneuroma/paraganglioma
Somatostatin	#	#	carcinoid/ganglioneuroma/paraganglioma
Substance P	—	+	paraganglioma
Vasoactive intestinal peptide	+	+	ganglioneuroma/paraganglioma
Nouronal marker			
Myelin basin protein	—	+	ganglioneuroma
Neurofilament protein			
160kDa	#	#	ganglioneuroma
200kDa	#	#	ganglioneuroma
Nouron specific enolase	#	#	carcinoid/ganglioneuroma/paraganglioma
S-100 protein	#	#	ganglioneuroma
Growth factor			
basic fibroblast growth factor	+	+	ganglioneuroma/paraganglioma
Others			
Leu7	+	#	carcinoid/ganglioneuroma/paraganglioma
Tyrosine hydroxylase	—	+	ganglioneuroma/paraganglioma

Score: + ; slightly immunoreactive, # ; highly immunoreactive

— ; no immunoreactivity

by thin, delicate, vascularized fibrous septa (Fig. 1b). The ganglioneuroma was composed of clusters of ganglion cells in which nuclei were frequently pyknotic and contained fibrous connective tissue matrix (Fig. 1c). The carcinoid consisted of areas in which neoplastic cells had formed ribbons, festoons and small glands in a manner indistinguishable from the usual type of carcinoid (Fig. 1d). Some of the carcinoid tumor cells and paraganglioma cells had intimately intermingled.

Histochemistry and immunocytochemistry

Table 2 shows the histochemical and immunocytochemical reactivities of the neoplasm. Reactivities of the latter type were scored as indicated in the footnotes to the Table 2. Both argentaffin and argyrophilic granules were observed in the cytoplasm of paraganglioma cells in both tumors. Positive cells, although not so numerous, were polygonal or fibrocytic and were located at the periphery of the

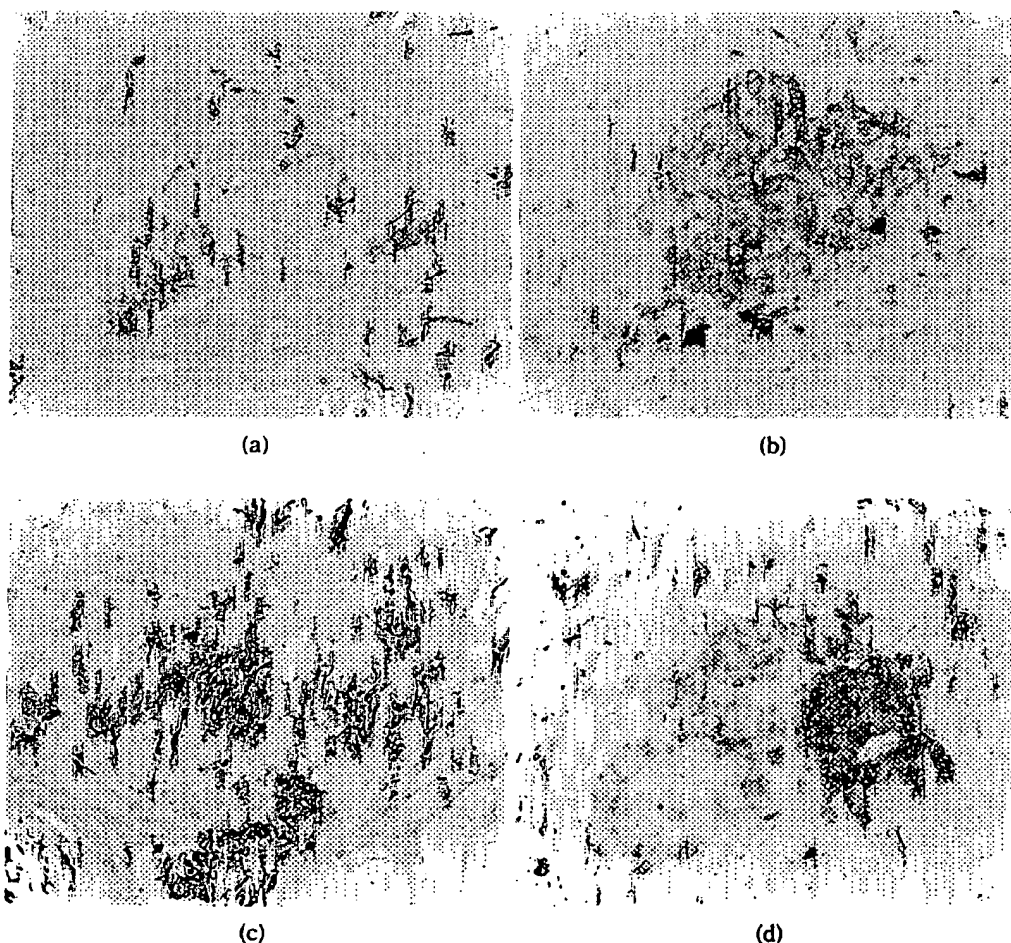


Fig. 2. Immunolabelled tumor cells with antibodies against ACTH (a), bombesin (b), CT (c) and CGRP (d) in the paraganglioma. a-d; $\times 200$.

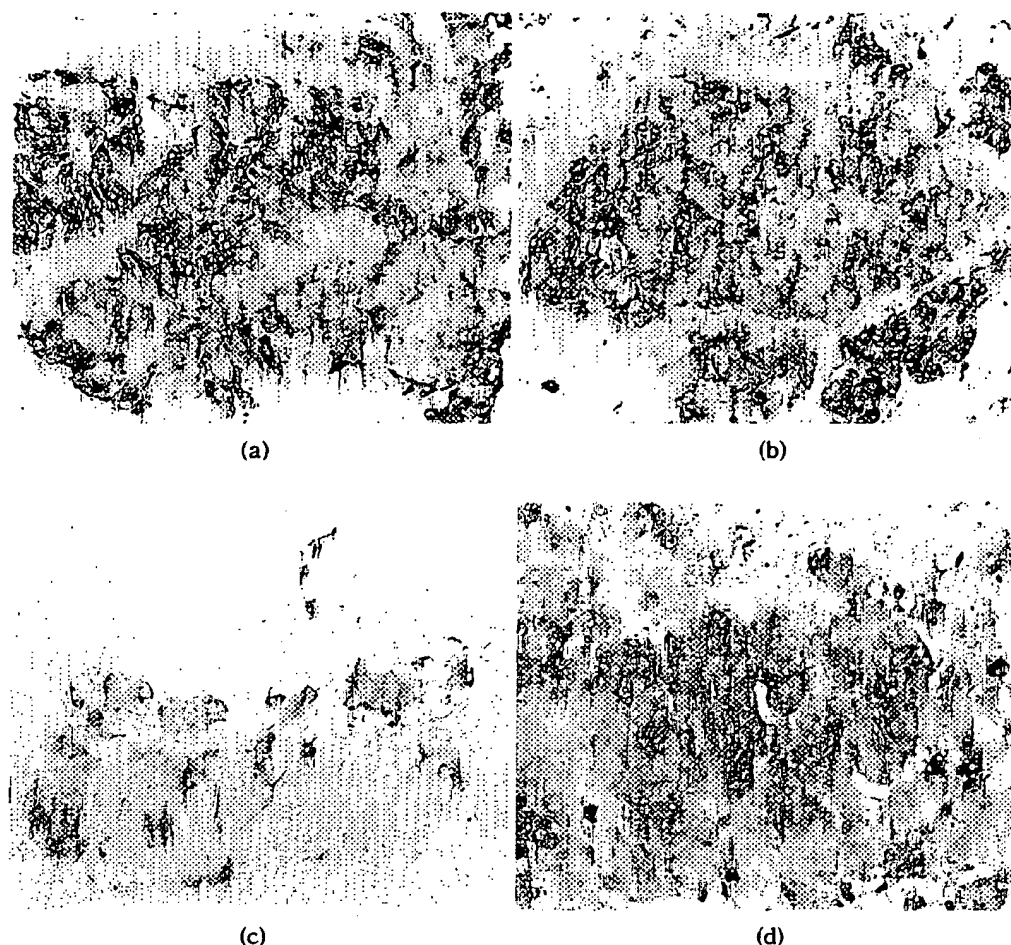


Fig. 3. Carcinoid tumor cells positively immunostained for human PP (a), and GRP (b); paraganglioma tumor cell stained positive for NPY (c) and EGC (d). a, c, d; $\times 200$, b; $\times 100$.

tumor nests (not shown). Positive immunoreactivity was detected to 12 peptides, including adrenocorticotropin (ACTH), bombesin, calcitonin (CT), calcitonin gene-related peptide (CGRP) (Fig. 2a-d), HPP, GRP, NPY (Fig. 3a-c), leu-enkephalin, PPP, somatostatin, substance P and vasoactive intestinal polypeptide (VIP). Positivity to neuroendocrine cell markers, such as chromogranin A, endocrine granule constituent (EGC) (Fig. 3d), Leu7 (Fig. 4a), tyrosine hydroxylase (Fig. 4b) and neuroendocrine marker, was found as well. Other proteins detected in the tumors included basic fibroblast growth factor (bFGF), although its reactivity was weak, myelin basic protein (MBP), 200 kDa (not shown) and 160kDa neurofilament protein (Fig. 4d), neuron specific enolase (NSE) and S-100 protein. The following immunoreactivities were also noted but only in case 2; these were GRP in paraganglioma and carcinoid, ACTH and bombesin in paraganglioma, MBP in fibers of ganglioneuroma, neuroendocrine marker in paraganglioma, and tyrosine hydroxylase in paragangli-

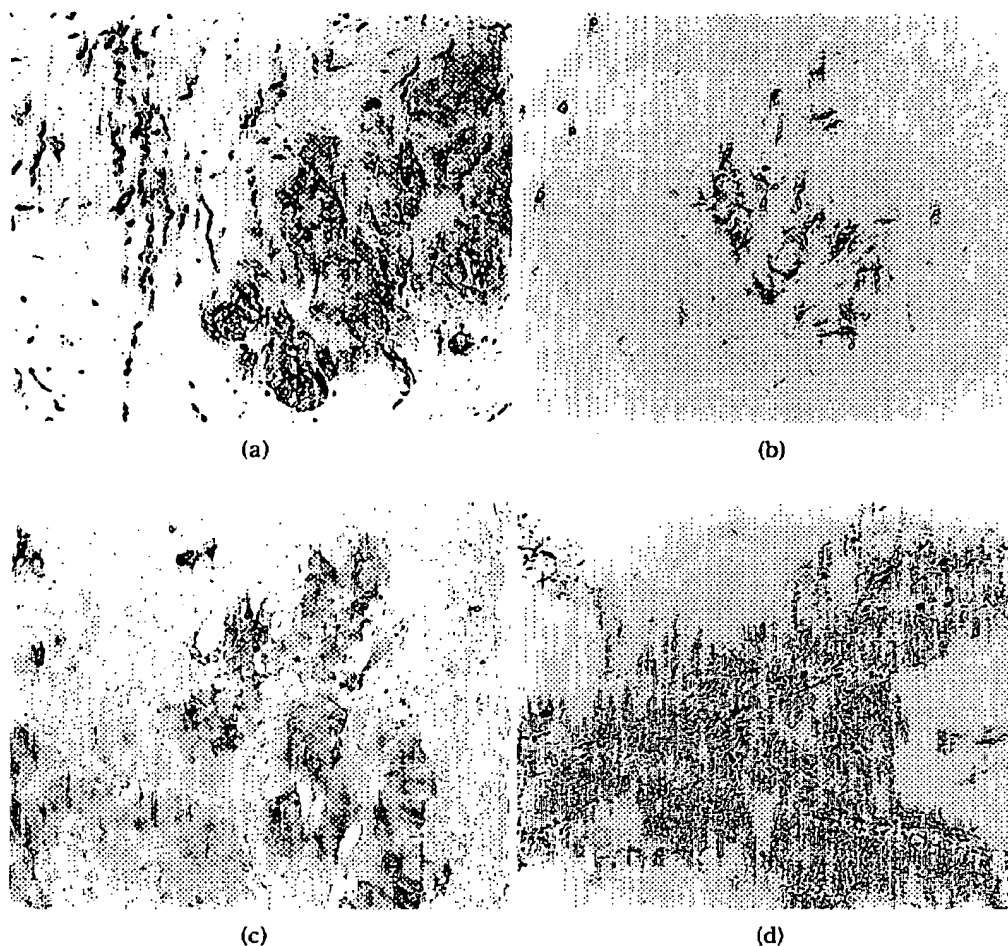


Fig. 4. Positive immunocytochemical staining of Leu7 in the ganglioneuroma (a), tyrosine hydroxylase in the paraganglioma (b), basic FGF in the paraganglioma (c) and NFP (160kDa) in the ganglioneuroma (d). a, b; $\times 100$, c; $\times 200$, d; $\times 40$.

oma. Among the immunoreactivities tested, CGRP, NFPs, Leu7, NSE, PPs, somatostatin and S-100 protein were frequently observed.

DISCUSSION

Gangliocytic paraganglioma was first reported by Dahl *et al.* in 1957¹⁶. A few years later in 1962, Taylor and Helwig described nine cases of nonchromaffin paraganglioma of the duodenum¹⁷. Since then, about 96 additional cases have been reported^{1,3-11,18-29}. Almost all of these cases demonstrated argyrophilia but not argentaffinity. Our two cases represent the first reported instances of both argyrophilia and argentaffinity, albeit within a limited area. Although the argentaffin method can be used to visualize serotonin storing cells, serotonin immunoreactivity was not present in our cases. Therefore, the significance of this argentaffin-positive

reaction is not clear, however, the possible origin of this tumor will be discussed later. Fifty-two reported cases were examined with respect to their immunohistochemical features, as stated earlier. Results^{2,4,5,7,9,29)} revealed that NSE, S-100 protein, NFP, PP, chromogranin A, somatostatin, leu-enkephalin, and protein gene product 9.5 were frequently detected in tumor tissue, whereas CCK, molluscan cardioexcitatory peptide, serotonin, gastrin, VIP, glucagon, insulin and substance P were only infrequently mentioned^{11,12)}. In the current study, we found many tumor cells which showed positive immunoreactivity for PP, somatostatin, leu-enkephalin, NSE, S-100 protein, NFPs, and chromogranin A as was the cases in the previous reports. In addition, CGRP, EGC, Leu7 and NPY immunoreactive tumor cells were found frequently in our two cases. Moreover, calcitonin, b-FGF, MBP and TH immunoreactivities were detected as well, although the frequencies were rather low.

The overall immunocytochemical features of the tumors presented seem to be similar to those of pheochromocytoma³⁰⁻³⁴⁾ and paraganglioma³⁵⁾, but the frequent detection of PP-like immunoreactivity in this tumor is a distinctive finding, since the usual form of paraganglioma or pheochromocytoma does not show this PP-like immunoreactivity. Further discussion related to this will appear later.

The origin of this tumor is still a matter of controversy because of histological complexity. However, on the basis of morphological and immunohistochemical analyses, several sources have been implicated. These include pluripotent stem cells residing at the base of intestinal glands³⁾, undifferentiated neural crest cells³⁾, multipotential cells of the embryonic celiac ganglion¹⁷⁾, residual paraganglionic cells and Meissner's plexus³⁶⁾, a paraganglionic vagal branch in the duodenum³⁷⁾, and embryonic neuroinsular complex⁵⁾ which was first described in the pancreas of sheep embryo by van Campenhout³⁸⁾. The first three of the postulated sources are unlikely, because pluripotent cells capable of differentiating to endodermal endocrine cells and neuroepithelial ganglionic cells have not been found in the duodenum. Residual paraganglionic cells and Meissner's plexus both seem to be an unlikely origin since paraganglionic tissue has not been described in the duodenal wall and the authors did not speculate why Meissner's plexus and residual paraganglion cells would act in concert to produce a neoplasm³⁶⁾. The last speculation seem to be rather feasible since sympathetico-insular complex does exist not only in the pancreas but also in the small bowel and appendix of mammalian embryos^{39,40)}. These endodermal-neuroectodermal complexes are thought to be composed of argentaffin cells that migrated from the crypts of Lieberkühn into the lamina propriae and submucosa, where they maintain intimate contact with nerve fibers and ganglion cells⁴⁰⁾. This finding explains the argentaffinity of the cells of this tumor. In human embryo of five-months gestation, Osaka and Kobayashi⁴¹⁾ described basigranulated endocrine cells intermingled with Schwann-like cells in the lamina propriae of duodenum and termed this association neuro-endocrine complex. This structure is thought to be homologous to the "complexe sympathico-insulaire³⁸⁾", later revised to the "complexe neuroinsulaire³⁹⁾", in mammalian embryo, and also to

the neuroendocrine complexes in the avian⁴²⁾ and rat stomach⁴³⁾. From these observations it is strongly suggested that this peculiar gangliocytic paraganglioma might be derived from these complexes exhibiting carcinoid and ganglioneuroma/paraganglioma patterns.

The frequent occurrence of the PP-like immunoreactive tumor cells in the duodenal paragangliocytic paraganglioma might not be a distinctive feature of this tumor since some duodenal carcinoid tumors have been reported to contain PP-like tumor cells⁴⁴⁾. In our tumors, PP-like immunoreactivity was found not only in the carcinoid but also in the paraganglioma. This suggests a possible transition of carcinoid tumor cells to paraganglioma-like cells, since the carcinoid cells in our cases were intimately entangled with paraganglioma cells suggesting this type of transition. More directly, the transformation of endocrine (carcinoid) tumor cells into neuron-like cells have been observed *in vitro*⁴⁵⁾. These authors reported that tumor cells spontaneously transform into cells with a neuron-like morphology and express neurofilament protein and synaptic vesicle membrane component.

In conclusion, our study indicates that duodenal gangliocytic paraganglioma exhibits many types of neuroendocrine immunoreactivity which are also shared with ganglioneuromas and paragangliomas occurring in sites other than the duodenum as well as with duodenal carcinoids.

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